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IN THE UNITED STATES PATENT OFFICE

In re Application of:

LEONARDO MARSILI et al.

Serial No. 913107

Filed: June 6, 1978

For: RIFAMYCIN COMPOUNDS

A F F I D A V I T

I, Aurora Sanfilippo, being duly sworn, depose and say:

That I am a citizen of Austria, residing in Milan (Italy), Via Sapri, n. 39;

That I received the degree of Doctor in Biological Sciences from the University of Milan in 1952;

That I have been employed by Societá Farmaceutici Italia S.p.A. since December 16, 1954 and that I am now employed in the FARMITALIA CARLO ERBA S.p.A. as Section Head of the Chemotherapy Department of the Research Laboratories. The Section I head for the FARMITALIA CARLO ERBA S.p.A. is responsible for the chemotherapeutic testing of the new products

synthetized by FARMITALIA CARLO ERBA S.p.A.;

That I have read the U.S. Patent Serial No. 4,086,225 and I have seen that the same covers and discloses four alkylderivatives only of piperidone: samples of said alkylderivatives have been supplied to me to be tested.

That I have read the U.S.Patent Application Serial No. 913,107 and I have seen that the radical R in the compound claimed in said application, when R is a linear or branched alkyl having 4 or 5 carbon atoms, can have several meanings: eight of such compounds have been supplied to me to be tested.

That I have carried out comparative trials both "in vitro" and "in vivo" between the alkyl derivatives according to Patent No 4,086,225 and the compounds according to patent application No. 913,107 as far as the antibacterial activity is concerned.

Since both groups of compounds are members of the group of rifamycins one of which is the rifampin which is therapeutically very successfully used as antitubercular agent, I have focused the antitubercular properties of these two groups of compounds.

That the results of my trials are set out in the following Tables A and B.

TABLE "A"

Compounds according to the U.S. patent No. 4,086,225 in which X and Z along with the C atom to which they are bonded, form a piperidine ring substituted on the N atom with the herebelow specified radicals.

Radical	A U C	Mycobacterium Tuberculosis H 37 R_v	
		M I C µg/ml	P D_{50} mg/Kg
$-CH_3$	14	0,005	5
$-C_2H_5$	9	0,01	10
n $-C_3H_7$	11	0,01	5
i $-C_3H_7$	11	0,005	5

TABLE "B"

Compounds according to the U.S. patent application No. 913,107 in which the radical R has the meaning herebelow specified

Radical R	A U C	Mycobacterium Tuberculosis H_{37} R_v	
		M I C µg/ml	PD_{50} mg/Kg
n-C_4H_9	21	0,001	5
i-C_4H_9	36	0,0012	1,25
sec.-C_4H_9	26	0,005	3,7
n-C_5H_{11}	16	0,0012	3
$CH_3-\underset{CH_3}{CH}-CH_2-CH_2-$	21	0,00005	not tested
$CH_3-CH_2-CH_2-\underset{CH_3}{CH}-$	26	0,00005	not tested
$(C_2H_5)_2-CH-$	58	0,0012	2,5
$CH_3-\underset{CH_3}{CH}-\underset{CH_3}{CH}-$	36	0,005	5

The MIC on Mycobacterium Tuberculosis $H_{37}R_v$ was determined with the method of twofold serial dilutions in Dubos-Albumin Medium (Difco) inoculated with about $2X10^5$ cell/ml. After 8 hours incubation at 37°C, the MIC was recorded as the minimal concentration able to inhibit any visible growth.

The PD_{50} was tested in mice experimentally infected with Mycobacterium Tuberculosis $H_{37}R_v$ (2 LD_{50} i.v.). The treatment generally started 3 days after infection. The drugs were administered orally once a day, 5 days/week for 7 weeks. The PD_{50} was calculated at the end of the experiments (90 days) as the dose able to protect from death 50% of the infected animals.

The A U C (the area under the plasmatic curve) was calculated on the basis of the plasma levels obtained in mice treated by oral route with the drugs at the dose of 50 mg/Kg. The plasma levels were determined microbiologically on Sarcina lutea (Agar plate diffusion) 1/2, 1, 2, 4, 7 hrs after the treatment.

It can be observed that the compounds according to Table B (in which the Radical R includes 4 or 5 carbon atoms) are substantially better than those according to Table A having a Radical with less than 4 carbon atoms.

Indeed the M I C of the compounds according to Table B have values ranging from the same magnitude down to 100 times lower than those of the products according to Table A. Moreover the

PD_{50} of the compounds of Table B may be 1/4 lower than that of the products of Table A.

Finally the plasmatic curves A U C of the compounds of Table B are much higher (in the case in which R is $(C_2H_5)_2$-CH- the A U C is 4 times higher) than those of the parent products of Table A.

Further deponent sayeth not.

Republic of Italy
Province of Milan
City of Milan
Consulate General of the
United States of America
} ss

Aurora Sanfilippo

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Before me personally appeared Aurora Sanfilippo known to be the person described in the above affidavit, who signed the foregoing instrument in my presence, and made oath before me to the allegations set forth therein as being under oath, this 9th May, 1979.

Consul of the
United States of America